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A snapshot of the rumen protozoa: Protozoal contribution toward carbohydrate metabolism

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Context

Protozoa, along with fungi, represent the rumen eukaryotome and account for up to 50% of microbial biomass[1]. Nonetheless, they are often overlooked. It is established that the rumen protozoa play a significant role in carbohydrate metabolism yet few of these enzymes have been identified and characterised[2]. Enzymes previously isolated from the rumen microbiome have shown great potential for application in industry (biodiesel manufacture, the food industry, washing detergents etc.) suggesting a wealth of untapped, novel activity may be found in the rumen eukaryotes.

Study Aim

To explore the activity of the rumen protozoa using metatranscriptomic techniques.

Fig.1: Separation of protozoa from rumen fluid in pear-shaped, 1L burette.

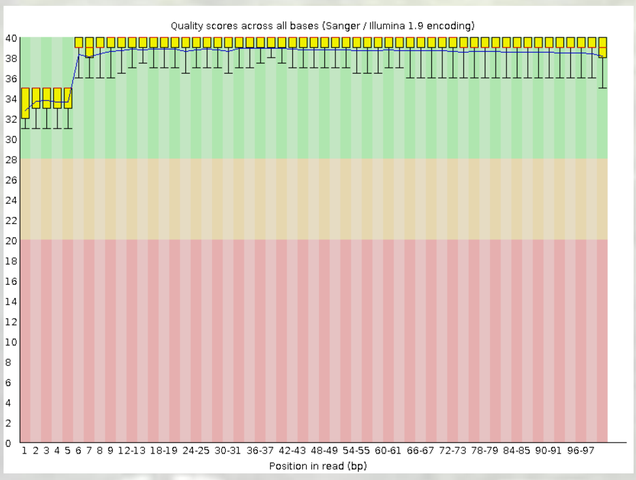

Fig.2: Graphical representation of quality after trimming. Central red line: Median; Yellow box: Inter-quartile range; upper and lower whiskers: 10% and 90%; blue line: Mean; Y-axis = quality score.

Table.1: Number of sequences through out bioinformatic processing and analysis

Data	No. sequences
Raw data	3,277,915
Trimmed data	3,125,731
Contigs Assembled	9,101
Predicted genes	2,505
Annotated genes	968
No. Reads aligning to contigs	402,226

Materials and Methods

Rumen samples were taken from three fistulated, non-lactating Friesian-Holstein cows and pooled. Protozoa were separated by addition of glucose (Fig 1) and subjected to several washes using Coleman's buffer and centrifugation. RNA was extracted (FastRNA Pro Soil-Direct kit), PolyA purified (Poly(A)Purist MAG kit), DNase treated (TURBO DNase) and reverse transcribed using SuperScript III Reverse transcriptase. The resulting cDNA was prepared for sequencing using the Nextera DNA Library Preparation kit and sequenced using an Illumina HiSeq 2500 rapid run.

Sequence data was handled using the Galaxy platform, first the data was quality checked (Fast QC) then trimmed (Trimmomatic) and quality checked again. Trinity was then used to assemble transcripts and TransDecoder used to identify coding regions (Table.1). The sequences were then annotated using the EggNOG gene Mapper online tool then applied to BowTie2 and FeatureCounts to give an expression profile. BLAST searches were also performed using existing protozoal glycosyl hydrolase sequences.

References:
[1] Belanche. A, Abecia. A, Holtrop. G (2011), *J. Anim. Sci.* **89**: 4163-4174.
[2] Qi. M, O'Toole. P, Barboza. P. S (2011), *PLOS One*, **6**(5).
[3]Bera-Maillet *et al.,*(2005), *FEMS Microbiol. Lett*, **244**(1): 149-159.
[4]Morgavi *et al*., (1994), *Microbiology*, **140**: 631-636.
[5]Coleman *et al*., (1980) *Adv. Parasit.* **18**: 121-173.

Results

- Overall, the majority of transcription was occurring in "Molecular processes and signalling" (Fig.3).
- In the "Metabolism" group, 72% of no. reads were those involved in carbohydrate transport & metabolism (Fig.3).
- Glycosyl Hydrolase family 5 and polysaccharide-active enzymes were both ranked in the top 20 most expressed enzymes overall (Fig.4).
- The protozoa also express pectin- and chitin-active enzymes (Fig.4).

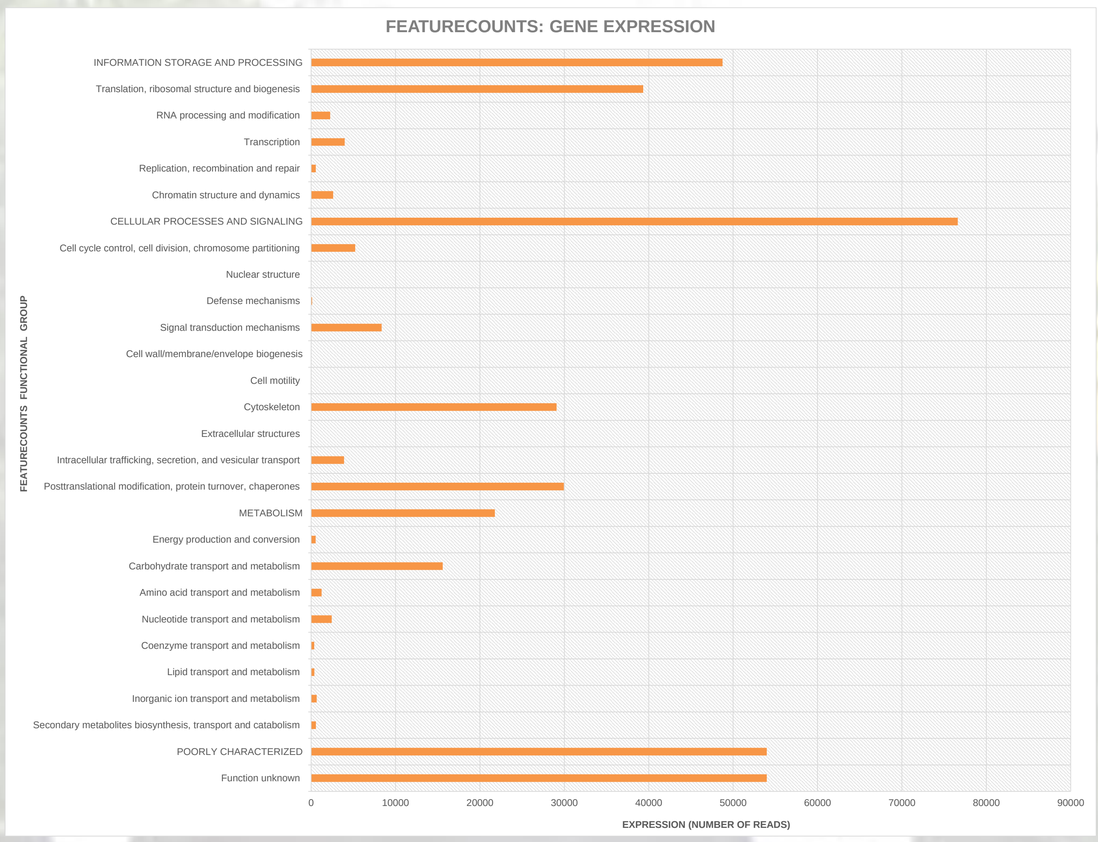

Fig.3: Graphical representation of FeatureCounts results giving an expression profile (number of reads) for the rumen protozoa.

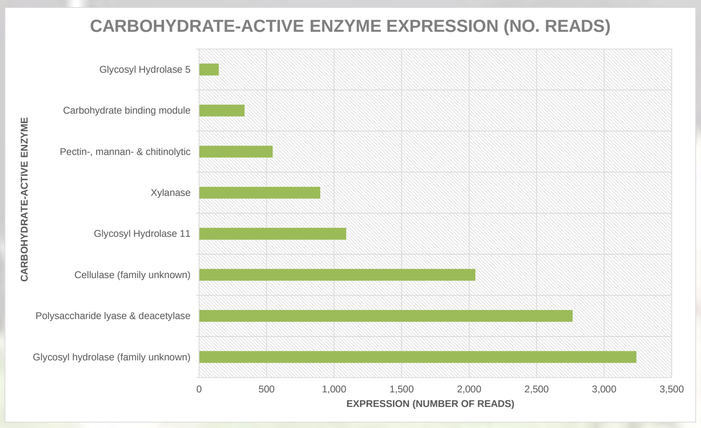

Fig.4: Graphical representation of FeatureCounts results for the carbohydrate-active enzymes (number of reads) of produced by the rumen protozoa.

Discussion

Whilst it is well established that the rumen protozoa contribute significantly to carbohydrate breakdown in the rumen, a metatranscriptomic approach has not yet been applied. This snapshot shows that the rumen protozoa produce a diverse range of enzymes and are well-adapted to thrive in their carbohydrate-rich environment. Polysaccharide deacetylases & lyases act on polysaccharides of plant, animal and microbial origin. In addition to the break down of plant material, these enzymes may also function in cell wall digestion of engulfed rumen bacteria. The abundance of uncategorised glycosyl hydrolases and cellulases is some what expected, although one may have anticipated more expression of xylanases[3].

Of interest, is the expression of both chitinases and pectinesterases/pectate lyases. It is likely that chitinases are produced to aid in the digestion of rumen fungi engulfed by protozoa and is likely to have been horizontally acquired from the rumen bacteria (similarity was shown to *Butyrivibrio* sp. and *Eubacterium* sp. chitinases)[4]. Alternatively, little is known as to why the rumen protozoa produce pectin-active enzymes as the breakdown of this compound does not support their survival or growth[5]. This study is being built upon by the metatranscriptomic analysis of the rumen protozoa over time in which we hope to provide support for the data presented here as well as new novel insights into these eukaryotes.